EXHIBIT 16

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Effect of Clozapine-Quetiapine Combination Therapy on Weight and Glycaemic Control

Preliminary Findings

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Abstract

Objective: The purpose of this open-label, non-randomised, 10-month, retrospective comparative study was to assess changes in weight and diabetes status for patients initially treated with clozapine who developed diabetes and who were then switched to clozapine-quetiapine combination therapy.

Methods: Sixty-five clinic charts were reviewed. All patients were from long-term care facilities. Bodyweight data were collected for this group of 65 randomly selected schizophrenic patients who were on clozapine initially (200 to 800 mg/day for 6 months) and then had quetiapine ('Seroquel') added to their therapy. Clozapine dosages were reduced as quetiapine was added proportionally: 25% of the clozapine dose was changed to quetiapine, using a ratio of exactly 1mg clozapine to 2mg of quetiapine. The quetiapine dosages ranged from 200 to 800 mg/day. This means that each patient received 6 months of clozapine therapy followed by 10 months of combination treatment with clozapine-quetiapine. Weights were recorded monthly, and diabetes status was also performed for patients who developed the condition during clozapine monotherapy.

Results: Changes in weight and the status of diabetes were determined in patients switched from a 6-month clozapine therapy to the 10-month combination clozapine-quetiapine treatment. All changes were statistically significant ($p < 0.001$). Use of this combination therapy in the management of weight gain and diabetes resulted in a 100% satisfactory response. All 65 patients showed weight loss ranging from 0.22 to 10.5kg (0.5 to 23lb) [mean 1.8kg (3.98lb)] after the first month of combination therapy, and the improvement continued through the study duration (10 months). Marked total weight loss ranged from 0.45 to 18.6kg (1 to 41lb), with a mean loss of 4.2kg (9.2lb) over the 10-month study period. 20% of patients (13 patients) who developed diabetes during the 6-month clozapine monotherapy showed significant improvement of disease status with addition of quetiapine. Compliance with medication was 100% and no significant adverse events were observed. The most common adverse event reported by patients was drowsiness. However, this did not contribute a valid reason for discontinuation of clozapine-quetiapine therapy and could be

corrected by dosage adjustment at any time of the report of this adverse effect by patients.

Conclusion: An unexpected, yet welcome, clinical effect of quetiapine is its apparent propensity to induce weight loss and improve glycaemic control in patients who gain weight and develop diabetes on clozapine therapy. The results of this retrospective study support the safety and tolerability of clozapine-quetiapine combination therapy.

Antipsychotic drugs can cause pronounced weight gain. This phenomenon can be regarded as a pharmacologically-induced adverse event and every effort must be made to prevent or vigorously treat it.[1] Clozapine is an atypical antipsychotic agent considered to have superior efficacy for patients with treatment-resistant psychosis.[2] No other atypical antipsychotic agent has been reported to be as effective as clozapine to date. Nonetheless, long-term administration of clozapine markedly influences eating behaviour and increases bodyweight in humans.

It is well known that weight gain is a serious undesirable effect of clozapine therapy, but the mechanism of clozapine-associated weight gain remains uncertain. Discussing neuroleptic-associated weight gain, Brady[9] noted that the mechanism is likely to be multifactorial. Possibilities include drug effects on serotonergic, anticholinergic and histaminic neurotransmitter systems, in addition to effects on endocrine and metabolic functions.

The complication of weight gain can result in noncompliance and a consequent return of psychotic symptoms.[3] Such an outcome can assume major clinical importance in the management of chronic schizophrenia, where maintenance of psychological and social well-being is dependent on regular administration of antipsychotic drugs.

Moreover, many patients with schizophrenia suffer from obesity,[4-6] which is associated with excessive rates of morbidity and mortality;[7] obesity is well recognised to be associated with an increased risk of morbidity from such conditions as diabetes, cardiovascular disease and locomotor disorders.[8] Particularly in these patients, additional weight gain is to be avoided.

Quetiapine ('Seroquel') is one of the most novel antipsychotic agents developed with the benefit of recent research. Quetiapine is an atypical drug for the treatment of schizophrenia or a related psychotic or schizoaffective disorders. Based on preclinical and recent clinical studies, quetiapine appears to have a pharmacological profile similar to that of clozapine without many of the latter drug's serious adverse effects, including weight gain and the development of diabetes.

The current retrospective study was undertaken to determine whether coadministration of clozapine and quetiapine could prevent the significant adverse effects of weight gain and development of diabetes experienced by schizophrenic patients taking clozapine only.

Patients and Methods

Study Participants

The target population consisted of all schizophrenic patients who were resident in Chicago's long-term care facilities. They were men and women ≥18 years of age who met DSM-IV criteria for schizophrenia and schizoaffective disorder. Those who demonstrated weight gain and/or developed diabetes during 6 months' treatment with clozapine monotherapy were eligible for the study.

Patients were receiving clozapine monotherapy and switched to clozapine-quetiapine. We evaluated changes in weight during clozapine monotherapy and clozapine-quetiapine combination therapy and status of diabetes in those developing it during the clozapine treatment time.

The study protocol and consent forms were

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approved by the local institutional review board. Written informed consent was obtained from each participant before the start of the study.

Methods

We employed an open-label, non-randomised design using retrospective chart review to identify patients and obtain data. Bodyweight data were collected for a group of 65 randomly selected schizophrenic patients who gained weight, and 13 (20%) of whom developed diabetes, while being treated with clozapine for 6 months and who were then switched to combination clozapine-quetiapine therapy. Clozapine dosages were 200 to 800mg per day. Clozapine was tapered up to 25% of the current dose and quetiapine was added proportionally: 1mg clozapine was substituted for 2mg of quetiapine. The quetiapine daily dosages ranged from 200 to 800mg.

Weight was recorded at baseline, monthly and at the conclusion of the study. Each patient was weighed monthly during the last 10 months of combination clozapine-quetiapine therapy and patients' diabetes status was determined concurrently by recording monthly blood glucose levels.

During the period of clozapine monotherapy, results of routine chemistry examinations revealed marked hyperglycaemia for the 13 patients (20%) who developed diabetes. Serum glucose levels were noted to be 0.36 to 0.85 mg/L, with a mean of 0.675 mg/L. Long-term control of hyperglycaemia was assessed by measuring glycosylated haemoglobin (HBA_{1c}) in fasting patients. Levels of HBA_{1c} were significantly higher than normal for patients who developed diabetes during clozapine monotherapy.

The onset of a response to clozapine-quetiapine combination therapy was defined as the initial appearance of clinical improvement with regard to significant weight loss and noticeable improvement of diabetes status.

Each of the 13 patients with diabetes began a regimen of regular insulin and a diabetic diet. Three patients discontinued hypoglycaemic agents and were placed on a regular diet. During the first 5 to 6 months of combination clozapine-quetiapine therapy, insulin requirements decreased and insulin was later discontinued. A regimen of the oral hypoglycaemic drug glibenclamide (glyburide) at 3 to 4 mg/day was started. Patients who showed a rapid resolution of all symptoms were placed on a regular diet.

Our primary goal was to show significant weight loss during the combination therapy. The second aim was to show that diabetes status could be improved during the combined clozapine-quetiapine therapy.

Statistical Analyses

Statistical analyses were performed on data from the intent-to-treat population, which comprised all 65 patients given the study medication. The statistical significance of improvement from baseline in both weight gain ($p < 0.001$) and effect on blood glucose levels ($p < 0.0001$) was calculated by paired t-tests. Patients were monitored monthly for a response to treatment and adverse effects. Data from all those who reported adverse events as well as improvement in the primary targeted symptom (weight loss and improvement of diabetes during this time) were tabulated in medical records.

Results

All 65 patients (100%) who commenced taking combination clozapine-quetiapine therapy showed significant weight loss and improvement in diabetes status during the period of combined clozapine-quetiapine therapy.

Weight Loss

At baseline, weight ranged from 59.5 to 125kg (131 to 275lb) [mean 104kg (229.2lb)]. During clozapine monotherapy, across all patients, the mean weight gain was 6.5kg (14.3lb; 6.25%) for the period of 6 months.

Marked changes in bodyweight were observed when patients started treatment with clozapine-quetiapine combination therapy. The quetiapine

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dose at 1 month ranged from 200 to 800mg per day. The individual weight loss ranged from a minimum of 0.23kg (0.5lb) after the first month of treatment to a maximum of 18.6kg (41lb) at the conclusion of the study [mean 9.4kg (20.75lb)].

All the changes in bodyweight were statistically significant ($p < 0.001$). All 65 patients showed weight loss ranging from 0.23 to 10.5kg (0.5 to 23lb), with a mean loss of 1.8kg (3.98lb), after the first month of combination therapy. Subsequent monthly losses were 1.8kg (3.98lb), 1.796kg (3.96lb), 1.456kg (3.21lb), 1.12kg (2.47lb), 0.966kg (2.13lb), 0.68kg (1.50lb) 0.635kg (1.40lb), 0.408kg (0.90lb), 0.318kg (0.70lb) and 0.227kg (0.50lb).

The improvement continued throughout the study to the end-point (10 months). Marked total weight loss ranged from 0.45 to 18.61kg (1 to 41lb), with a mean loss of 4.2kg (9.2lb) over the 10-month study period.

Diabetic Status

Twenty percent of the 13 patients who developed diabetes during clozapine monotherapy showed significant clinical and laboratory improvement of diabetes while taking clozapine-quetiapine. Weight gain in this group of patients ranged from 3.2 to 24.1kg (7 to 53lb) [mean 8.5kg (18.69lb)] during clozapine therapy. Thirteen patients who developed diabetes due to clozapine showed significant weight loss, with a mean loss of 1.75kg (3.84lb) after the first month of combination treatment and 4.68kg (10.3lb) at the conclusion of the study.

During clozapine therapy, patients showed significant increases of up to 10 to 15% in the HBA_{1c} level (monthly mean increase = 1.34%). HBA_{1c} levels returned to normal (<7%) at the end of the study (monthly mean decrease = 0.78%): results of routine chemistry examinations at the end of the 10-month treatment period revealed normalisation of blood glucose levels, resulting in a rapid decrease in insulin requirements and/or discontinuation of insulin treatment and starting of a regimen of an oral hypoglycaemic agent. Three patients who discontinued hypoglycaemic agents were placed on a regular diet and remained metabolically stable.

Positive results were assessed in terms of normalisation of blood glucose levels, discontinuation of insulin therapy, switch of patients to oral hypoglycaemic drug and placement of patients on a regular diet. According to our data, results of a laboratory examination revealed a normalisation of serum glucose levels in three of our patients, which is valid proof of improvement of diabetes and metabolic stabilisation.

Overall, our data demonstrated that no adverse behavioural changes occurred during the 10-month study period. No patients stopped therapy because of drowsiness; this was corrected by adjusting the dose. Compliance with medication was 100% and no significant adverse events were observed.

Discussion

The multiple clinical studies and reports from different researchers demonstrate significant weight gain in a group of schizophrenic patients during clozapine treatment. In spite of the considerable efficacy of clozapine, increased appetite, craving for sweets and weight gain are commonly cited by patients as their primary reason for discontinuation of the treatment.

The mechanism of clozapine-associated weight gain remains uncertain, as does the cause of hyperglycaemia associated with high doses of clozapine.

The first report of severe insulin-dependent hyperglycaemia precipitated by clozapine therapy in a patient with a previously unremarkable medical history was introduced by Kamran et al. in 1994.[10] According to his report, the sustained hyperglycaemia, which required insulin therapy and diet modification, completely resolved following discontinuation of clozapine, but he continued 'we do not know whether clozapine alone or the combination of clozapine, benztropine, and ranitidine was responsible for the hyperglycaemia'.[10]

The majority of patients in our current study were on valproate semisodium (divalproex sodium). Thus, the possibility that drug combinations

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may have affected the metabolism of one or more agents, resulting in altered drug levels and impaired glucose metabolism, must be kept in mind.

Diabetic ketoacidosis associated with clozapine treatment was also reported by Koval et al.[11] The author described a history of diabetes for a patient who did not have elevated serum glucose levels previously. This patient developed diabetes 6 months after initiation of clozapine treatment and was admitted to an intensive care unit in a comatose condition. She initially required insulin treatment. Clozapine treatment was discontinued slowly, and her insulin requirements decreased and insulin was later discontinued. This case also demonstrates that clozapine may precipitate insulin-dependent diabetes in some individuals. Further studies are necessary to investigate the relationship between clozapine therapy and blood glucose regulation.

Quetiapine is a recently introduced antipsychotic drug. In its pharmacological profile, quetiapine resembles other atypical antipsychotic agents with the exception of possible weight gain. An unusual clinical effect of the drug is its apparent propensity to induce weight loss, which could be a cause of the improvement of diabetes during combination clozapine-quetiapine therapy. There are obvious clinical implications arising from the propensity of an effective antipsychotic drug to produce weight loss as well as cause improvement in, and in some cases resolve, diabetes, leading to discontinuation of insulin or other hypoglycaemic drugs.

A great deal of work remains to be done with quetiapine, in particular to elucidate its mechanism of action and to determine the optimal dosage and length of treatment in combination with clozapine.

The current retrospective analysis was done to determine whether coadministration of clozapine and quetiapine could attenuate the significant unpredictable adverse effects of weight gain and development of diabetes during clozapine monotherapy.

This study may contribute to the discovery of novel therapeutic approaches to the treatment of refractory schizophrenic patients with clozapine and quetiapine without serious adverse effects such as significant weight gain and development of diabetes, which can occur during clozapine monotherapy.

To date, no study had compared clozapine monotherapy and combination clozapine-quetiapine therapy. Future studies should focus on larger sample sizes to corroborate the findings of the current study. Furthermore, a double-blind, randomised, prospective study would have been preferable. Limitations of this current retrospective analysis are non-standardised administration, uncontrolled concomitant therapy, non-randomised assignment and data censoring.

Conclusion

The current study demonstrated that the combination of clozapine and quetiapine had a significant, positive effect on weight and glycaemic control.

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